

FIG. 9



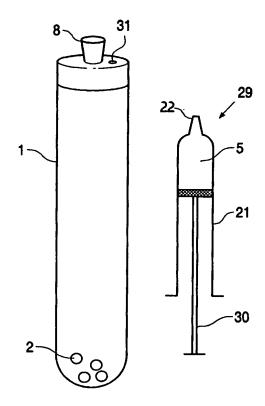


FIG. 11

Figure 12. Strategies followed in the given examples

Figure 12.1 Ex vivo monitoring of immune response against tetanus toxoid.

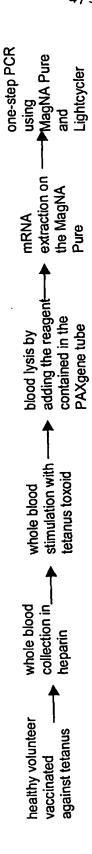
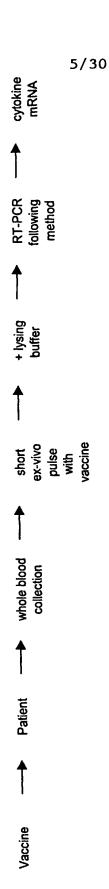


Figure 12.2 Strategy followed in example 3.

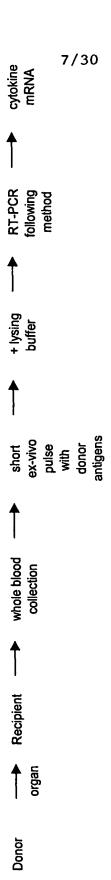


6/30



Figure 12.3 Strategy followed in example 4

Figure 12.4 Strategy followed in example 5.



8/30

PBMC

Blood

Figure 13.1

9/30

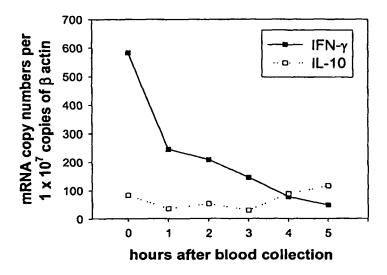


Figure 13.2

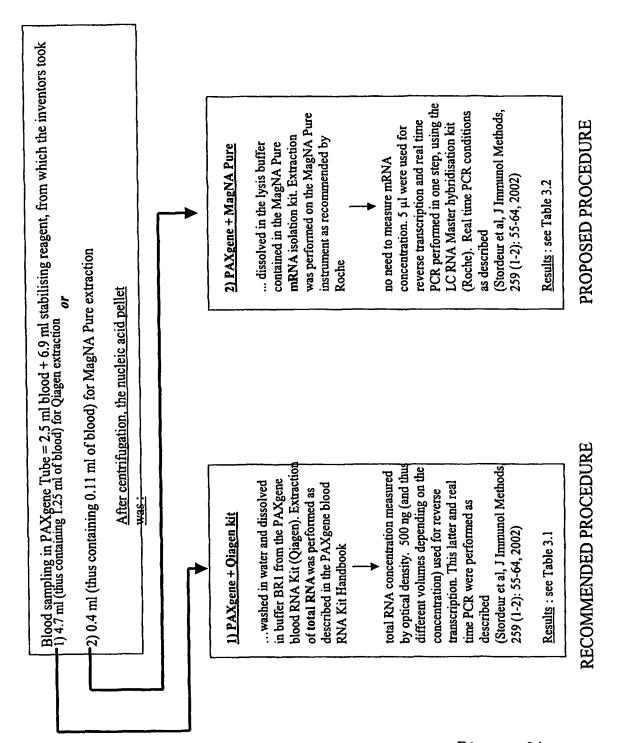


Figure 14



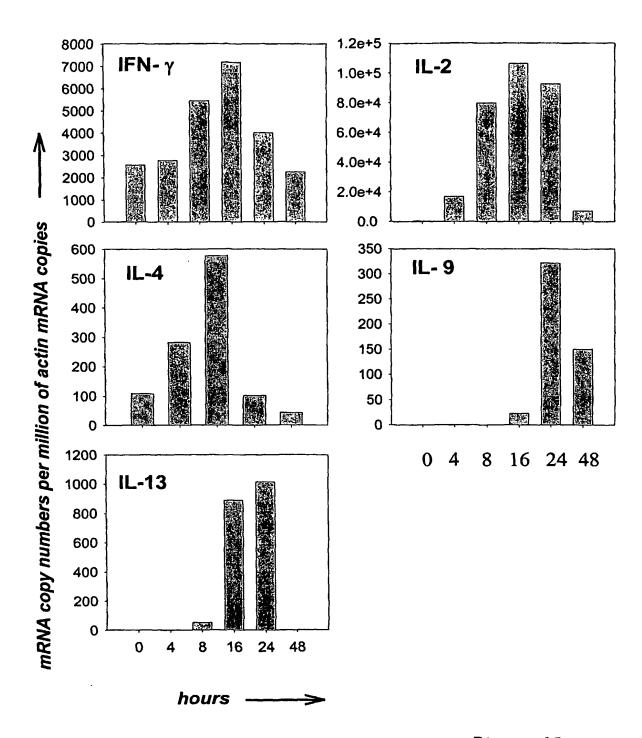


Figure 15

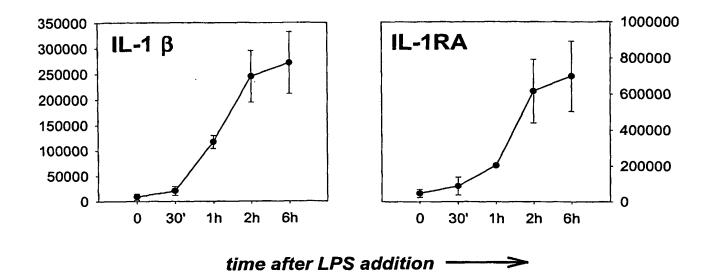
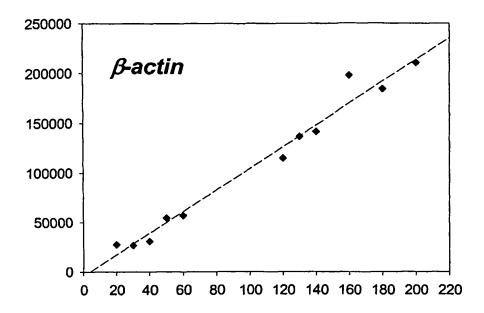


Figure 16





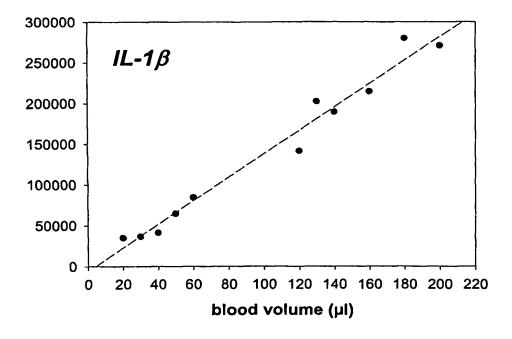


Figure 17

14/30

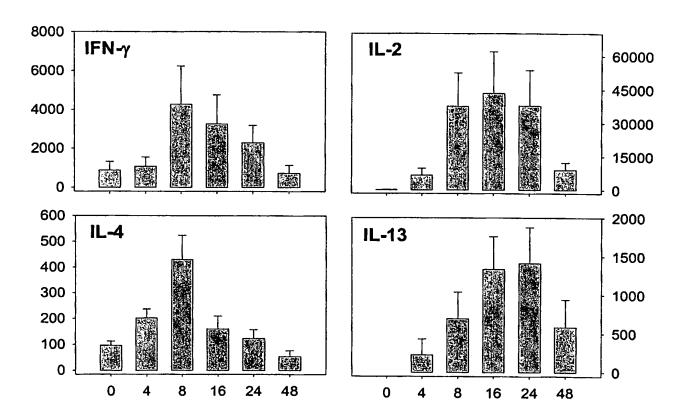


Figure 18

15/30

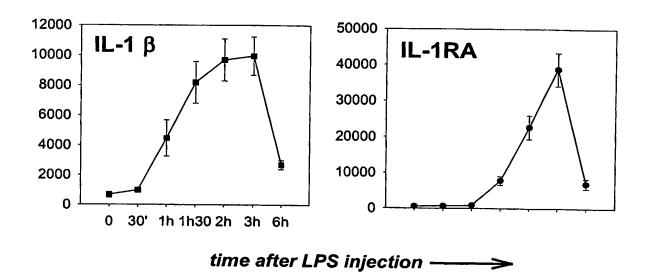


Figure 19



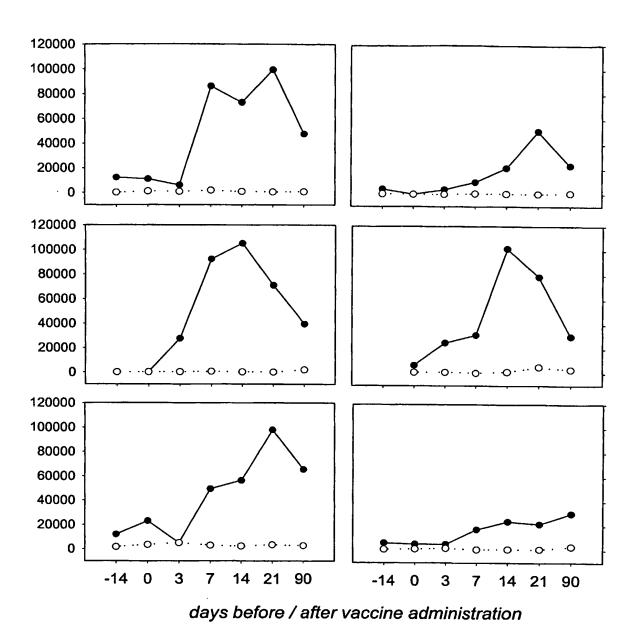


Figure 20

17/30

Procedure to analyse blood cytokine mRNA expression

- Whole blood sample incubation in the presence the tested potential immuno-modulator (antigen, allergen, cell, xenobiotic,...)
- Blood cells lysis and mRNA stabilisation using the reagent contained in the *PAXgene* tube
- Automated mRNA extraction and preparation of the reaction mixture for one-step RT-PCR using an automated device (Magna Pure instrument Roche Diagnostics)
- Quantification of cytokine mRNA levels by real-time PCR using the Lightcycler instrument (Roche Diagnostics)

18/30

Automated mRNA extraction and reagent mix preparation on the MagNA Pure: direct correlation between starting cell number and found copy number

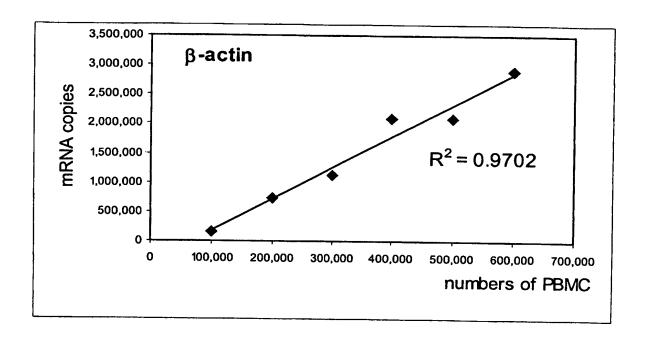


Figure 22

19/30

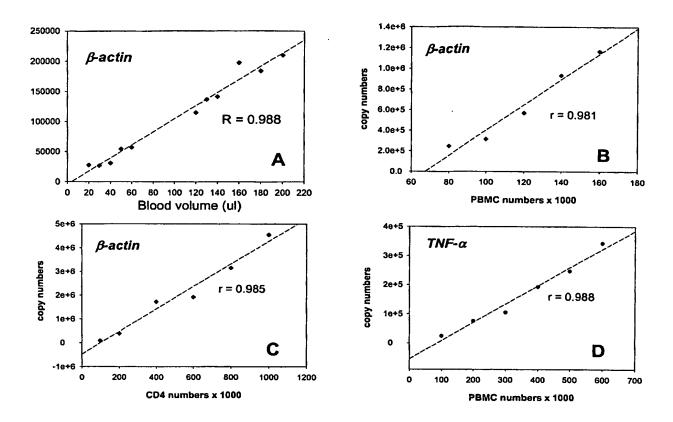


Figure 23

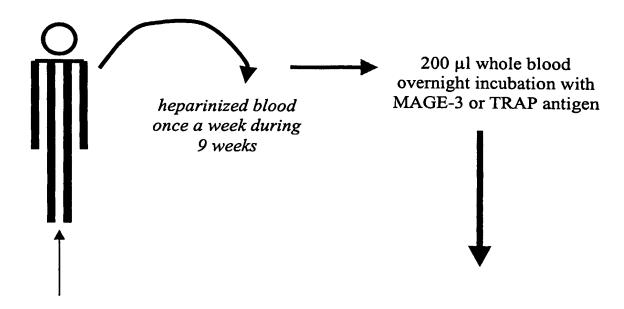
20/30

Immune monitoring in cancer immunotherapy

- 7/99: melanoma of the right scapula. Excision
- 8/01: SC M+ arm, back, abdomen, right testis
- 4/02: right orchidectomy
- 4/02: pre-vaccine check-up
- 5/02: initiation of the vaccine program

21/30

In vitro stimulation of whole blood to assess immune response to MAGE-3

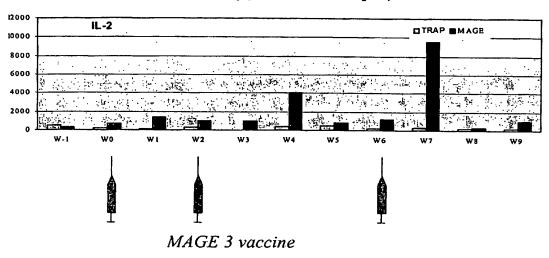


Patient #3 received 3 injections of anti-MAGE-3 vaccine (MAGE-3 + adjuvant)

mRNA extraction and RT-PCR using the Magna Pure and the Lightcycler

IL-2 mRNA in whole blood following MAGE-3 vaccination in Patient #3

IL-2 mRNA (copy numbers per million of β-actin mRNA copies)



23/30

In vitro stimulation of whole blood: evaluation of immune response to allergen

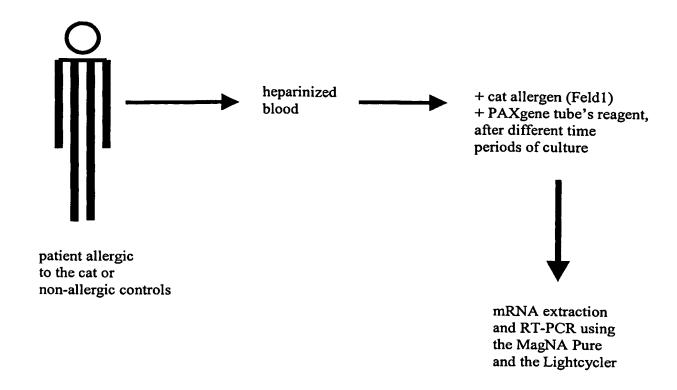
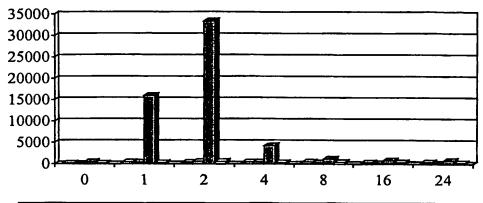


Figure 27

24/30

In vitro stimulation of whole blood : evaluation of immune response to Feld1



■ non allergic + allergen (1) ■ non allergic + allergen (2) ■ allergic + allergen □ allergic, no allergen

Figure 28

In vitro stimulation of whole blood: dose-response to Feld1

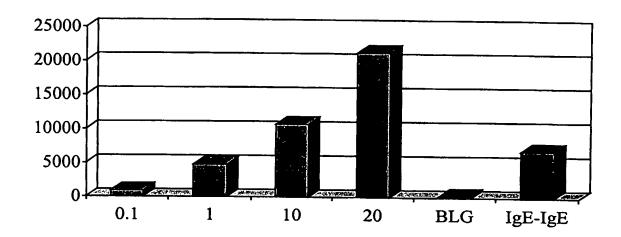
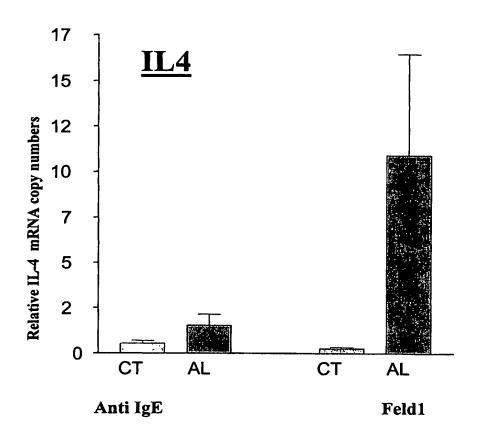


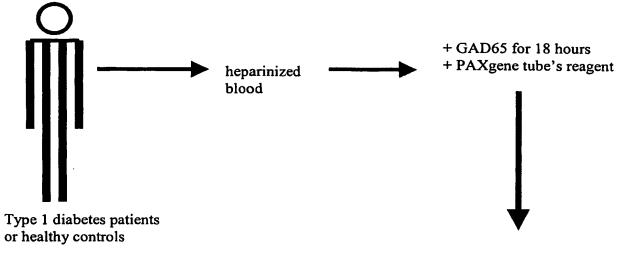
Figure 29

IL-4 mRNA levels after whole blood stimulation with Feld1 are higher in allergic patients compared to healthy controls



B HEALTHY
PATIENTS ALLERGIC TO CAT

In vitro stimulation of whole blood : assessment of T cell response to GAD65



mRNA extraction and RT-PCR for IL-2 mRNA using the MagNA Pure and the Lightcycler

28/30

In vitro stimulation of whole blood: assessment of T cell response to GAD65

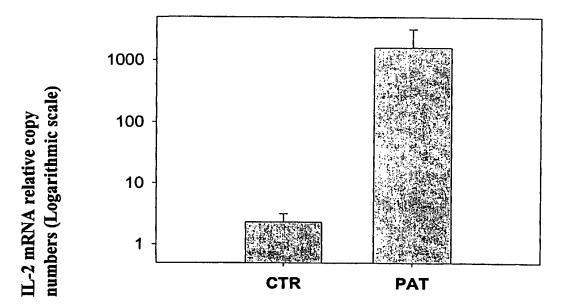


Figure 32

Monitoring of alloreactive immune response: quantification of IL-2 mRNA in a whole blood + dendritic cells system

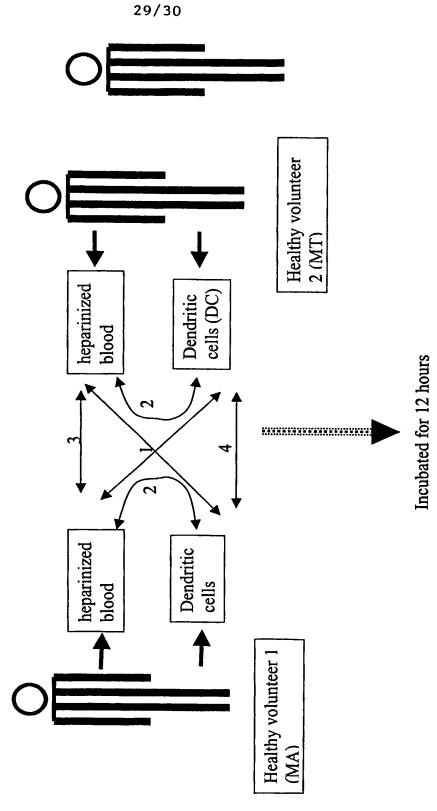


Figure 33

30/30

Monitoring of alloreactive immune response: quantification of IL-2 mRNA in a whole blood + dendritic cells system

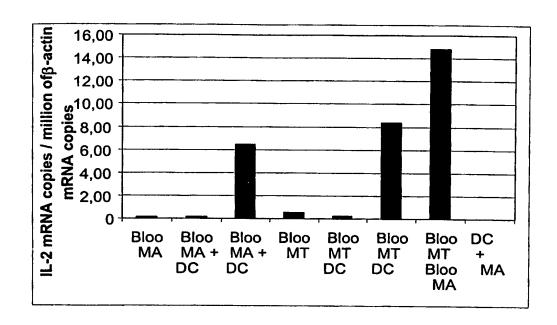


Figure 34